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In D-loop: 40 years of mitochondrial 7S DNA

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ABSTRACT

Given the tiny size of the mammalian mitochondrial genome, at only 16.5 kb, it is often surprising how little we know about some of its molecular features, and the molecular mechanisms governing its maintenance. One such conundrum is the biogenesis and function of the mitochondrial displacement loop (D-loop). The mitochondrial D-loop is a triple-stranded region found in the major non-coding region (NCR) of many mitochondrial genomes, and is formed by stable incorporation of a third, short DNA strand known as 7S DNA. In this article we review the current affairs regarding the main features of the D-loop structure, the diverse frequency of D-loops in the mtDNAs of various species and tissues, and also the mechanisms of its synthesis and turnover. This is followed by an account of the possible functions of the mitochondrial D-loop that have been proposed over the last four decades. In the last section, we discuss the potential links of the D-loop with mammalian ageing.

1. Mitochondrial genome organisation

Nearly all eukaryotes maintain a multi-copy mitochondrial genome independently of the nuclear genome. Human mitochondrial DNA (mtDNA) was first sequenced in 1981, and this sequence was subsequently revised eighteen years later (Anderson et al., 1981; Andrews et al., 1999). Human mtDNA is a double-stranded circular DNA molecule of 16,569 bp, and encodes thirteen polypeptides, all of which are core components of Complexes I, III, IV and V of the respiratory chain. These thirteen proteins are transcribed and translated by a dedicated machinery in the mitochondrial matrix, with human mtDNA also encoding 22 tRNAs (one tRNA per amino acid, with two each for serine and leucine) and two ribosomal RNAs for this purpose. Human mtDNA shows remarkable compactness and contains almost no intergenic regions, with two pairs of protein-coding regions even overlapping. The two strands of mtDNA are denoted as the 'heavy' (H) and 'light' (L) strands; an historical annotation based upon their distinct base compositions, which results in different buoyancies on caesium chloride gradients (Fig. 1). Both the heavy- and light-strands of mtDNA are transcribed as long, polycistronic molecules, with transcription initiated from the heavy-strand promoters (HSPs) and light-strand promoter (LSP), respectively. The mechanism by which mtDNA replicates is an unresolved issue, and three models are currently proposed. The strand-displacement model, devised in the early 1970s, and the RITOLS model, proposed in 2006, both define one major origin of replication for each DNA strand (Clayton, 1982; Yasukawa et al., 2006). These are denoted O_H (the H-strand origin) and O_L (the light-strand origin). O_H is located in the major non-coding region (NCR), while O_L is located approximately two-thirds of the way around the molecule in a cluster of five tRNAs. These origins divide the genome into roughly two-thirds and one-third sections, with the larger portion denoted the 'major arc', and the smaller portion denoted the 'minor arc' (Fig. 1). Similar to bacterial chromosomes, mitochondrial DNA is organised into tightly-packed nucleoprotein complexes called nucleoids. Nucleoids are readily visible using fluorescence microscopy, where they appear as scattered punctate foci (Satoh and Kuroiwa, 1991). Nucleoids are found associated with the inner mitochondrial membrane, but can also freely diffuse through the mitochondrial network (Albring et al., 1977; Brown et al., 2011). The most thoroughly characterised nucleoid protein is the high-mobility group box protein TFAM (Parisi and Clayton, 1991). TFAM is a core transcription factor in human mitochondria, and is also capable of non-selectively binding and inducing negative supercoiling of mtDNA, greatly compacting the genome (Ngo et al., 2011; Rubio-Cosials et al., 2011). TFAM exists in sufficient abundance to coat the entire mitochondrial genome, and is therefore

Abbreviations: CSB, conserved sequence block; D-loop, displacement loop; HSP, heavy-strand promoter; HVS, hypervariable segment; LSP, light strand promoter; OXPHOS, oxidative phosphorylation; NCR, non-coding region; RITOLS, ribonucleotide incorporation throughout the lagging strand; SDM, strand displacement mechanism; TAS, termination-associated sequence.

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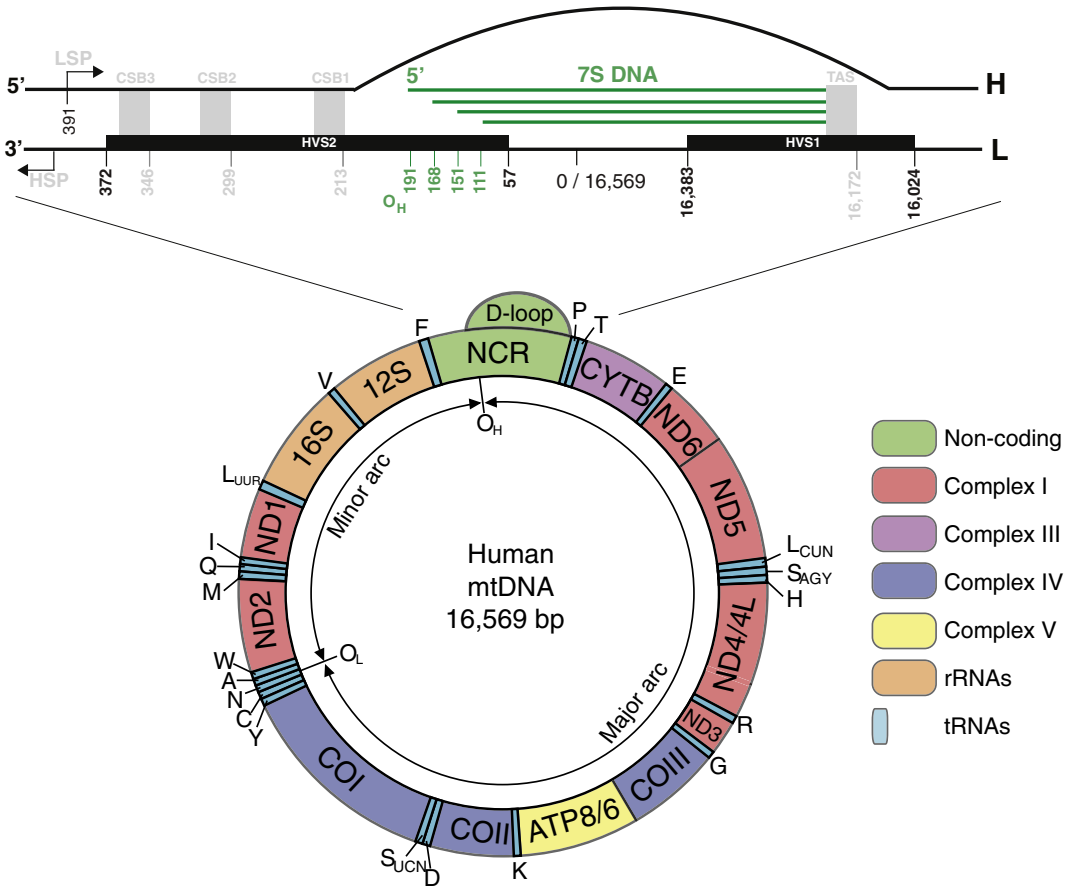

Fig. 1. (Top) The detailed structure of human mitochondrial NCR and D-loop. The position of the main 5′ ends of 7S DNA (green) are indicated. HSP and LSP — heavy- and light-strand promoter, respectively. CSB — conserved sequence block, TAS — termination associated sequences, HVS1 and HVS2 hypervariable regions 1 and 2. (Bottom) Organisation of the human mitochondrial genome. Human mtDNA is a circular molecule of 16,569 bp, and encodes 13 polypeptides, as well as the 22 tRNAs and 2 rRNAs necessary for their translation. The classical replication origins O_H and O_L divide the genome into roughly two-thirds and one-third regions, which are designated the "major arc" and the "minor arc", respectively. Most of the *cis*-elements required for mitochondrial transcription and replication are contained within the non-coding region (NCR). Many mtDNA molecules incorporate a third, linear strand that covers a large portion of the NCR, forming a triple-stranded structure known as the D-loop.

considered to be the core component of the nucleoid (Kukat and Larsson, 2013; Takamatsu et al., 2002).

Aside from the 13 mtDNA-encoded polypeptides, around another 1000 proteins constitute the mitochondrial proteome, and are required for proper mitochondrial function (Pagliarini et al., 2008). These proteins are all encoded by the nucleus, are translated in the cytosol and imported into mitochondria. This includes, but is not limited to: all other structural subunits of the oxidative phosphorylation (OXPHOS) system, OXPHOS assembly factors, the replication and transcription machineries, all ribosomal proteins and translation factors, Krebs cycle enzymes, proteins for β-oxidation, iron–sulphur cluster assembly, haem biosynthesis, and pyrimidine biosynthesis.

2. Features of the D-loop structure in the major mtDNA non-coding region

The major non-coding region (NCR) of human mtDNA spans approximately 1.1 kb between the mt-tRNA genes of phenylalanine and proline. The NCR contains the HSP and LSP promoters for transcription of the heavy- and light-strands, respectively, as well as the classical origin of heavy-strand replication, O_H. A large part of the NCR often incorporates a linear third DNA strand of approximately 650 nt, forming a stable D-loop structure (Fig. 1). This additional strand is called 7S DNA, based upon its sedimentation properties. The terms 'NCR' and 'D-loop' are frequently used interchangeably in the literature, although this is not necessarily appropriate as the D-loop region does not span the entire NCR, and only a proportion of mtDNA molecules contain a D-loop at any given time. The D-loop region extends from around O_H (at the 5′ end of 7S DNA) to the termination-associated sequence (TAS) close to the gene for tRNAPro (at the 3′ end of 7S DNA) (Doda et al., 1981). The 5′ end of 7S DNA may lie at any one of a number of defined sites in humans, meaning that 7S DNA exists as a family of molecules of slightly different sizes (Crews et al., 1979; Fish et al., 2004; Kang et al., 1997) (Fig. 1). The D-loop was first identified in electron micrograph images of mouse and chicken mtDNA over forty years ago (Arnberg et al., 1971; Kasamatsu et al., 1971; Robberson et al., 1972). Stable D-loop structures appear to be a feature of many animal mtDNAs, having since been observed in other species including humans, rabbits, cows and *Xenopus* (Annex and Williams, 1990; Brown et al., 1978; Hallberg, 1974; Kasamatsu et al., 1971). The proportion of mtDNA molecules containing a D-loop at any given time ranges from around 10% in cultured human cells, up to around 90% in *Xenopus* oocytes (Brown et al., 1978; Callen et al., 1983; Hallberg, 1974) (Table 1). The formation and stability of the D-loop depend upon sequence elements within the NCR, and consequently the organisms mentioned above retain a broadly similar NCR layout. Comparisons of the NCR sequences of human and mouse mtDNAs originally revealed the presence of three 'conserved sequence blocks' (CSBs) located between LSP and O_H, referred to as CSB1, 2 and 3 (Walberg and Clayton, 1981). This analysis was later extended to include many more species, finding that CSB1 (which lies immediately upstream of O_H) is very well conserved, with CSB2 being only partially present in some species, and CSB3 being sometimes missing